doi:10.1017/S1431927611001528
Microsc. Microanal. 17 (Suppl 2), 2011

The Role of Oligomerization in Poliovirus Polymerase Function

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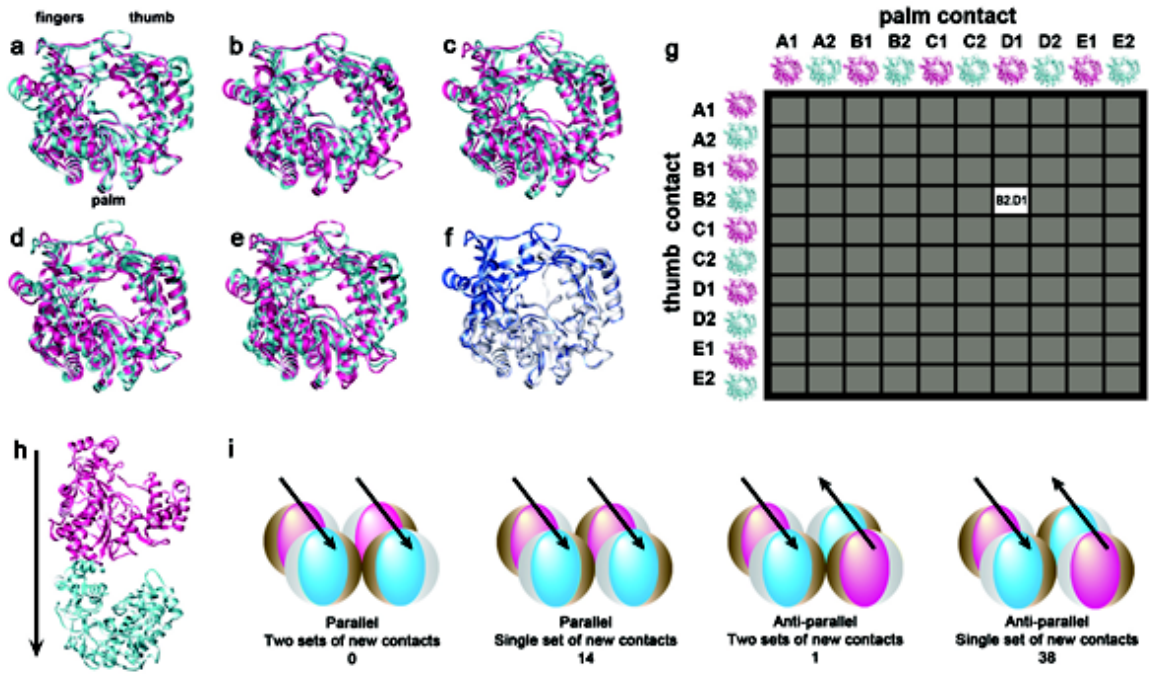
Macromolecular assemblies of poliovirus polymerase require both stability and mobility to support viral replication. An integrated biophysical approach using molecular dynamics modeling and energy minimization; site-directed mutagenesis; electron cryo-microscopy and cryo-tomography; image processing; and enzymatic activity assays is providing a detailed view of the oligomerization that promotes cooperative replication.

A replication platform is expected to assemble on host-cell vesicles. This replication complex would promote localization of enzyme, template, and substrate. Inclusion of two-dimensional arrays of poliovirus polymerase in such a replication 'factory' would allow for efficient RNA transfer and, therefore, cooperative enzymatic activity. A planar morphology appears to be mimicked in laboratory experiments, through assembly of wild-type polymerase into hollow tubes (rolled up two-dimensional sheets). Our helical reconstructions of polymerase tubes using electron cryo-microscopy data are now available for fitting of the newly modeled polymerase structures, and are being used to test, validate, and further refine the modeling data.

Additional experiments underway are providing additional constraints and validation for our emerging model of the polymerase replication platform. These include electron cryotomography of a mutant polymerase that assembles into layered sheets ('bricks') of protein. Each layer of this array is expected to include both Interface I and the second interface we are defining through studies of the wild-type polymerase tubes. Assembly of the bricks is stabilized by a third interaction not present in wild type protein oligomers, and results in more highly-ordered protein arrays. We expect that macromolecular assemblies of wild type polymerase on the surface of synthetic liposomes will be less ordered than this mutant, but will again recapitulate the interactions present in both wild type polymerase tubes. As expected, electron cryo-microscopy of polymerase-containing liposomes shows limited array formation, likely due to the fluid nature of lipid membranes and the absence of additional replication factors that are present in virus-infected cells.

Incorporation of poliovirus polymerase data from these diverse biophysical methods, in combination with activity assays from each of these macromolecular assemblies, is leading to an atomic model of poliovirus replication. This emerging model is based on our improved understanding of both the dynamic range of individual polymerase subunits and the subunit-subunit interactions that support the formation and stabilization of two-dimensional polymerase arrays.



Building and testing a polymerase oligomer model

Normal mode analysis (http://www.igs.cnrs-mrs.fr/elnemo) was used to determine a range of conformational changes to mimic native conformations of poliovirus polymerases within macromolecular assemblies.

Panels **a-e** show structures from the five lowest energy models. These models were calculated using the solved crystal structures (panel **f**, pdb files 1rdr shown in gray and 1ra6 in blue) as starting models. The two conformations with the largest r.m.s. deviation are shown from each normal mode in panels **a-e**.

g. A dimer was created using each pair of conformations.

h. One such dimer is shown, D1.B2 from panel **g**

i. Orientations for lattice formation from two dimers into a lattice

Results from computational modeling were tested empirically using site-directed mutagenesis, turbidity measurements, and electron microscopy.

https://doi.org/10.1017/S1431927611001528 Published online by Cambridge University Press